REMARKS

Claims 1-13 are pending and claims 1, 2, 5, 6 and 8-11 have been allowed. Claims 3, 7, 12 and 13 have been rejected. Claim 4 has been objected to and claim 10 has been cited for informalities. The disclosure has been objected to for various informalities and typographic errors.

In response, applicants amend claims 3, 4, 7, 10 and 12. Applicants also amend the specification to correct informalities and typographic errors, as suggested by the Examiner.

Applicants thank the Examiner for allowing the claims 1, 2, 5, 6 and 8-11.

Reconsideration and allowance of the remaining claims earnestly are requested.

Written Description Rejections

On pages 2-3 of the Office Action the Examiner has rejected claims 12-13 under 35 U.S.C 112, first paragraph. In response, applicants amend claim 12 to overcome the rejection. Allowance is therefore requested.

Definiteness Rejections

On pages 3-4 of the Office Action, the Examiner has rejected claims 3 and 7 under 35 U.S.C 112, second paragraph. Applicants respectfully disagree with the Examiner and refer to the following:

> For definiteness, a claim need only reasonably apprise those skilled in the art of the utilization and scope of the invention. *Hybritech, Inc. v. Monoclonal Antibodies,* 231 USPQ 81, 94-95 (1986). Words are to be given their plain meaning as understood by the person of ordinary skill in the art, particularly given the limitations of the English language. *See* MPEP §§ 707.07(g); 2111.01. Claims are to be given their broadest reasonable interpretation consistent with applicants' specification. *See* MPEP § 2111. In sum, in order to reject the claims on definiteness grounds, it is incumbent on the examiner to show how and why the skilled person having applicants' specification would

not be apprised of the invention by the language-at-issue. The rejections are discussed below.

The Examiner has rejected claim 3 for using the term "marker complex" and stated that "the use of the term 'marker complex' as being compromised within the primer sequence elements in Claim 3 seems to contradict its description as being carried by the hybridizing sequence so that it may reach the target structure (see p.5, lines 8-10)." Applicants respectfully disagree with the Examiner. Neither claim 3 nor the specification describes a marker complex "within" the primer sequence elements. The specification clearly defines a 'marker' (see specification, for example, p.5, lines 8-27) and how a 'marker complex' is built (see the specification, for example the paragraph bridging pages 5-6). Applicants also note that a skilled artisan would understand how a marker complex with a hybridizing element can bind a target structure during hybridization. For further clarity, applicants amend claim 3.

Concerning claim 7, the Examiner states that "the claimed invention [is] impossible to practice" because the claim misses an important step . Although applicants believe that claim 7 is clear, applicants nevertheless amend claim 7 for better clarity.

Withdrawal of the rejections and allowance are solicited.

Claim Objections

On page 9 of the Office Action the Examiner has objected to claim 4 and stated that the claim lacks proper dependent form for further limiting claim 1. The applicants respectfully disagree. Claim 4 limits claim 1 by providing a specific sequence of the SK primer element. However, for additional clarity, the applicants amend claim 4. Reconsideration and allowance are solicited.

Drawings

The Examiner has requested clarification of the presence of "MCS SK" within the depiction of SK-PH I in Figure 1 (b). In response, the applicants specify that "MCS SK" indicates the location of the MCS-SK site on the plasmid generally, wherein "SK-PH I" is inserted. Applicants refer to the specification (see, for example, the bridging paragraph on pages

14 and 15) for a detailed description of Figure 1 (b). For further clarity, applicants amend Figure 1(b) by deleting the word "MCS SK" from the drawing.

Regarding the description of the Figure 5, this information has been moved into the section "Brief Description of the Drawings" of the specification, as suggested by the Examiner.

Miscellaneous

On pages 9-10 of the Office Action the Examiner has objected to claim 10 because of informalities. In response, Applicants amend claim 10 per the Examiner's suggestion and have followed the nomenclature indicated in claim 2. Allowance is solicited.

Specification

On page 4 of the Office Action the Examiner has made several suggestions. In response, applicants have amended the specification, excluding the claims as suggested. The specification is revised and a substitute specification (excluding the claims) is provided in compliance with 37 CFR 1.52(a) and (b) based on PCT publication PCT/DE00/00116 (WO 00/40736), which does not constitute "a new matter".

On page 8 of the Office Action other informalities have been objected to. In response, applicants have amended the specification to correct grammatical errors and as published under WO 00/40736, which does not constitute "a new matter."

CONCLUSION

In view of the foregoing, Applicants respectfully request the Examiner to withdraw the rejections against claims 3, 7, 12 and 13. The Examiner is invited to contact the undersigned attorney to resolve any issues, in order to expedite the prosecution of the application.

Respectfully submitted,

12/16/02
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Copy of Marked-up Claims and Specification

Claims:

Amend the claims as follows:

3. (twice amended) The plasmid of claim 1, wherein the SK primer sequence elements comprise a marker that binds to a target structure during hybridization [complex].

4. (twice amended) The plasmid of claim 1, wherein the SK primer sequence element [comprises the sequence (SEQ ID NO: 5):] is

5' - GATCCACTAGTTCTAGAGCG-3' (SEQ ID NO: 5).

7. (twice amended) A method of detecting a target structure by analytical electron microscopy, comprising the steps: [of adding]

a) providing the plasmid of claim 1;

b) adding a marker to the plasmid to form a plasmid-marker complex;

c) binding the plasmid-marker complex to the target structure; and

d) imaging the bound complex by electron microscopy.

10. (twice amended) A test kit for use in electron microscopy comprising:

[-]a) host *E. coli* JM110 bacterial cells suitable for replicating the plasmid of claim 1; and

[-]b) a single-stranded plasmid comprising at least one of 2[x], 7[x], 14[x], 21[x], and 27[x] repetitive SK primer sequence elements.

12. (amended) A reagent for electron microscopy comprising a pBluescript KS(+) derivative that comprises a multiple SK primer sequence element; and a SK oligonucleotide with end modification by a detectable element, wherein the element is detectable by electron microscopy.

Specification:

Insert the following as the first sentence of the specification:

--This application receives priority from application PCT/DE00/00116, filed January 07, 2001, which claims priority to German Patent Application No. 199 00 511.7, filed January 07, 1999, the entireties of which are hereby incorporated by reference.--

Page 1, after the first sentence of the specification, as noted immediate above, insert:

-- BACKGROUND OF THE INVENTION --

Page 1, line 3, paragraph 1: Amend as follows:

The invention relates to [a series of new] plasmids [on the basis of] derived from pBluescript KS(+), comprising more than 1 SK primer sequence element, preferably 2, 7, 14, 21 and 27 repetitive SK primer sequence elements, and [the] their use [thereof]as [a]molecular-biological markers [for] in analytical electron microscopy.

Bridging paragraph pages 1-2: Amend as follows:

[Thus far,] M[m]ultiple labeling experiments have been carried out in electron microscopy by using gold grains of various sizes to be able to differentiate the different target structures in a single preparation. For example, one molecule type would be linked to gold grains having a size of 5 nm while the other would be attached to those having a size of 10-20 nm in a double labeling experiment to ensure that in a subsequent evaluation the different molecules can clearly be localized and distinguished from one another. Large gold grains (larger than 10 nm) are disadvantageous because they have reduced penetration capacity into the tissue and reduced coupling efficiency to the target molecule (Giberson, T.R., and Demaree, R.S.: The influence of immunogold particle size on labeling density. Microscopy Research and Technique, 27, 355-357, 1994). In addition, such a large structure can no longer be assigned clearly to the site of binding to the target structure, i.e. resolution capability is lost. If a triple labeling experiment was aimed at, these drawbacks would become particularly striking. Only what is called ferritin molecules, i.e. large protein units which contain hundreds of iron atoms in their centers and can be linked to

target structures, are an alternative to the gold grains. However, their electron density and [thus] their detectability under the transmission electron microscope is very poor so that their use has only proved feasible in rare cases.

Bridging paragraph pages 2-3: Amend as follows:

[In contrast thereto]On the other hand, florescence methods [which] enable triple [labeling,] and [even] quadruple labeling[,] without causing major problems [have existed in the field of] in optical microscopy[for some time now]. Electron microscopy [Since as regards the] with the existing labeling techniques [electron microscopy cannot] could not compete with optical microscopy[for the time being,]. Therefore, scientists [are] have been using optical microscopes [satisfied] with [the] comparatively poor resolution capability. [of optical microscopes before they accept the drawbacks accompanying the labeling technology in the electron-microscopic field.] The development of alternative labeling techniques for gold labeling would render electron microscopy more attractive because its [competitiveness as regard] advantageous labeling, [would be accompanied by a]provides a resolution capability over 100 times as good as that of optical microscopy. [As an alternative to the] The gold labeling method [using gold]for [the]conventional transmission electron microscopy[, which] is based on the electron density of the heavy metal gold[,] and there is a demand for alternative labeling methods for ESI. This technique utilizes interactions between beam electrons and the atoms in the preparation differing from those of conventional transmission electron microscopy. In principle, all of the elements can be detected specifically. This raises the number of elements in consideration for labeling methods. However, to establish alternative labeling methods, it is decisive to check detection limits for the elements in consideration. This means, in concrete terms, that information is required on the number of detectable element atoms per nm^2 area in the preparation. [Thus] Therefore, the detection limits of the ESI technique are relevant. [Thus far,] [these are o]Only a few [some] study[ies]-reports and vague indications on this parameter are available. Although the ESI technique is often used, no data on detection limits have been published to date.

Page 3, paragraph 1-2: Amend as follows:

Therefore, t[T]here is [thus] a demand for alternative labeling methods for electron microscopy. It should be possible to readily test and assess the detectability of such a marker complex.

[It is thus]Therefore, [the]an object of the present invention is to provide a method [possibility] of obtaining data[by means of which it is possible], to evaluate the prospects of the intended experiment with the element in question and/or the marker structure in question, before time-consuming cytobiological and molecular-biological experiments are carried out. Furthermore, the parameter for the detectable number of elementary atoms per unit area shall become measurable to obtain necessary [therefrom the] information [necessary] to establish EM labeling methods.

Page 4, lines 1-14: Amend as follows:

The reason why the above-mentioned preliminary tests are necessary is that [thus] so far no accurate limiting values of detectability have been known for the ESI detection of the various chemical elements. This is *inter alia* due to the fact that preparing a suitable test sample is not a trivial matter. Such a sample must have special properties. There must be regions in which the target element is available in a clearly defined amount. It must be possible to clearly detect these regions. The target element may not occur in the remaining regions. This problem has been reported [can be shown by means of the publication] by investigators [Golla, U. and Kohl, H. (Micron, 28:(5), 397-406, 1997)] who [using uranium as an example] tried to [document] record the resolution and detectability by means of grainy precipitates, using uranium as an example (see, Golla and Kohl, Micron, 28:(5), 397-406, 1997).

Page 4, between lines 14 and 15: Insert:

-- BRIEF SUMMARY OF THE INVENTION

In one aspect, the invention provides plasmids derived from pBluescript KS(+), comprising more than 1 SK primer sequence element, preferably 2, 7, 14, 21 and 27 repetitive SK primer sequence elements.

In another aspect, the invention provides methods of detecting a target structure by analytical electron microscopy, comprising: providing the plasmid as described herein; adding a marker to the plasmid to form a plasmid-marker complex; binding the plasmid-marker complex to the target structure; and imaging the bound complex by electron microscopy.

In yet another aspect, the invention provides a test kit for use in electron microscopy comprising: host *E. coli* JM110 bacterial cells suitable for replicating the plasmid as described herein; and a single-stranded plasmid comprising 2, 7, 14, 21, and 27 repetitive SK primer sequence elements.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 5. Figure 5 shows, as an outline, an overall diagram of a plasmid (Solid circular line) that contains 16 repetitive SK primers (16 small solid circles), to which ESI markers bind; and, in greater detail, a diagram of two 20-nucleotide long repetitive SK primer fragments (the lower strand) that are separated by a spacer of a 4-oligonucleotide long fragment (see a gap on the lower strand). ESI markers (the solid balls) are covalently bonded with the single-stranded oligonucleotides (upper strands). The oligonucleotides (upper strands) are bound by complementary base pairing (as a result of hybridization) of the repetitive SK primer fragments (the lower strand) on the plasmid.

DETAILED DESCRIPTION OF THE INVENTION --

Bridging paragraph pages 7-8: Amend as follows:

These[s] SKOPHs are preferably separated by the chromatography of unbound SKOs. This may be done by column chromatography, e.g. Amersham Pharmacia Biotech (Freiburg, Germany) offer column matrixes (e.g. sephadex or sepharose). The purified SKOPHs are then subjected to spreading. In this connection otherwise coiled DNA molecules are pretreated such that they are

stretched in solution and in this state are applied onto electron-microscopic small carrier nets coated with a thin sheeting, made visible by treatment with heavy metals and analyzed under a transmission electron microscope (TEM). If ESI analysis shall be carried out, heavy metal treatment should be dropped, since every element occurring in high amounts and/or high concentrations in the preparation interferes with, or makes impossible, the specific detection of the target element. The DNA rings are then distributed uniformly over the surface of the TEM preparation and are separate from one another. When [The] above-mentioned basic preconditions are [thus] met: the annular DNA is clearly evident, the SKOs are available in a more or less large number and are bound to the DNA, and there is (almost) nothing in between the DNA regions.

Page 9, at lines 12-13, paragraph 1: Amend as follows:

The repetitive sequences are arranged closely one behind the other and extend over about a third of the plasmid. These repetitive sequences render the test much more significant. The advantage of the above described plasmids consists in that 1 to 27 of the marker units can be accumulated so as to modulate the number of marker elementary atoms. When it is possible to show the labeled SKOPHs in differing spreading states from fully extended to coiled in the spreading preparation, the target elementary atoms, bound particularly to coiled DNA molecules, can i) be concentrated within a very confined space, ii) become localizable due to the uniformly fibrillary ring shape of the [DNA] bound- DNA [thereto], iii) be analyzed in defined but variable number, and iv) be in an otherwise element-free environment.

Bridging paragraph pages 9-10: Amend as follows:

It is [the] an objective of this test method to obtain reliable data on the minimum number of target element atoms per unit area necessary for ESI detection. At the same time, data are obtained on the individual detectability of the marker structure and because [due to]of the repetition, [thereof] it is also possible to obtain average weak element-specific signals, in particular, in DNA molecules available in the electron-microscopic preparation in a fully stretched manner. [Thus]Therefore, it can [already] be determined prior to a technically complicated use of a marker structure in medicine or biology whether optionally the number

or/and the concentration of the target elementary atoms must still be increased in the marker structure. [As experience shows, a]All of the plasmid states from stretched to considerably coiled are found in spreading preparations, in particular when the spreading process did not proceed in optimum fashion. This [usually undesired case] is of advantage in connection with the explained determination of elemental detection limit.

Page 10, Paragraph at line 16: Amend as follows:

The threshold values for the element-specific detection can be determined by the standard methods of elemental detection using ESI. For this, there is presently no other method. Therefore, [I]it can [thus]be [imagined] considered that this method is also of interest for the scientists who do not have in mind a biologically/medical use but are interested in the detection limits of any chemical elements other than those mentioned above. The precondition is that the target element is already present in the marker structure linked to the oligonucleotide in the highest possible concentration and in the greatest possible amount.

Page 12, Paragraph 2: Amend as follows:

The plasmid construction is stabilized by introduction into a dam⁻/dcm⁻ strain (preferably *E. coli* JM 110). JM110 is dam⁻/dcm⁻ and contains no other striking genotypic markers, which would clearly distinguish this strain from the other ones used, so that they can be employed as well. The repetitive plasmids according to the invention are introduced into the dam⁻/dcm⁻ strain according to standard methods (*cf.* Sambrook, J., Fritsch, E.F. and Maniatis, T.: Molecular cloning; A laboratory manual; Second edition, Cold Spring Harbor Laboratory Press (1989)). Surprisingly, a deletion of the directly repetitive elements is [thus] avoided during bacterial replication. It is [actually] known that the direct repeats or inverted repeats are lost during the replication in *E. coli*. [d]Dam/dcm strains are documented in the literature (*cf.* Marinus *et al.*, J. Bacteriol. 114 (3), 1143-1150 (1973)); however, stabilization of directly repetitive sequences, resulting therefrom, has never been described.

Page 14, Paragraph 2, section a) of the Figure 1 description: Amend as follows:

a) (SEQ ID NO:2) Diagram of pBl KS(+). pBl KS(+) was digested with the restriction enzymes Kpn I and BamH I for subsequent cloning. The restriction sites are marked by a finely broken line. The [MS] MCS fragment therebetween falls out.

Page 17, lines 4-13: Amend as follows:

[The below described m]Methods of producing the plasmids containing the repetitions are well known in the art [described in Sambrook, J., Fritsch, E.F. and Maniatis, T.] (see, for example, Sambrook *et al.*, Molecular cloning; a laboratory manual; second edition; Cold Spring Harbor Laboratory Press, 1989[)]; and [in] Current Protocols in Molecular Biology, [(]John Wiley and Sons, 1994-1998).[,] [t]These [below]techniques, including, for example, [such as]DNA replication, restriction endonuclease digestion, ligation, agarose gel electrophoresis, and PCR, are known to skilled artisans[being sufficiently known to, and mastered by, a person skilled in the art].

Bridging paragraph pages 17-18: Amend as follows:

A short oligonucleotide fragment is required for the construction of SK primer sequence elements in repetitive succession. It contains the SK primer sequence and restriction sites for carrying out cloning. For this purpose oligonucleotides complementary to one another were synthesized. These ss-DNA fragments were converted by hybridization into clonable ds fragments by juxtaposing the two complementary oligonucleotides in equimolar fashion in 10 mM Tris buffer. [Accomplished cloning is a control for the success.] A successfully obtained clone was used as a control. The resulting fragments were referred to as SK-PH I (SK primer hybrid I; fragment which was used for the SK primer replication of 2 - 6 SK primer sequences; see Ill. 1) and SK-PH II (SK primer hybrid II; fragment which introduced the seventh SK primer and the Eag I restriction site; see Ill. 2).

Bridging paragraph pages 18-19: Amend as follows:

The further cloning of plasmids with up to seven SK elements contained in equal orientation was time-consuming, since one clone from the last cloning run served in each case as a basis for the next cloning step. Correspondingly, the [midi]mini-prep-DNA of the select pBl 2x SK clone was again double-digested by BamH I/Kpn I and admixed with SK-PH I, ligated and transformed in *E. coli* XL1-Blue. Contrary to the strategy used for cloning pBl 2x SK, attention had then to be paid especially to an efficient double digest using BamH I and Kpn I. As shown in Ill. 1c, the restriction sites into which another SK-PH I fragment should be integrated, were only six base pairs apart from one another. Such a small distance between two restriction sites does not permit the simultaneous restriction of both restriction sites. Correspondingly, the restriction had to be carried out successively using the two enzymes. Cloning up to the plasmid pBl KS(+) 6x SK was carried out in this way.

Bridging paragraph pages 20-21: Amend as follows:

As compared to the first cloning steps, which resulted in pBl KS(+) 7x SK, the vector was not opened by two different enzymes (Kpn I/BamH I; see Ill. 1) but linearized by Not I. Therefore, an accumulation of religations had to be expected. In this cloning, a religation could not be counteracted by an insert concentration increased many times over (7x SK fragment), since the DNA blocks were phosphorylated at their 5' ends and uncontrollable oligomerizations of the insert DNA had to be expected. Therefore, the religations were reduced, or even suppressed, by dephosphorylating the vector. The DNA [as referred to as] pBlKS(+)7xSK, as described above, [thus far] is designated as the pBl 1x block below.

Bridging paragraph pages 21-22: Amend as follows:

Control digest with BamH I of several candidate clones showed that a complete 7x SK block had additionally been inserted. One of the clones was replicated for a [midi]mini preparation and the DNA was prepared. The sequence analysis from this [midi]mini preparation identified the complete and correct sequence of 21 SK primers including the functioning restriction sites which

were required for the next cloning run. The gel analyses were confirmed in this connection. This clone is referred to as pBl 3x block below. It served as a precursor for the next insertion run.

Bridging paragraph pages 22-23: Amend as follows:

One of the five equal clones was chosen and a sequence analysis was made using its [midi]mini-prepared DNA. Sequencing confirmed the result that the newly joined BamH I restriction site was deleted. The complete SK primer with intact BamHI restriction site of the last joined 7x SK block lacked. The result was [thus] a pBl KS(+) plasmid having 27x SK primers. The sequence of this clone is shown in figure 4.

In the Abstract:

Amend the abstract as follows:

The invention relates to [a] plasmids [which is characterized in that it is]derived from pBluescript KS(+), which [and]contains more than 1, preferably 2, 7, 14, 21 and 27, repetitive SK primer elements, and [to the use thereof for] their use in analytical electron microscopy.